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Synthesis of novel compounds based on reticuline scaffold for new drugs discovery

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Synthesis of Novel Compounds Based on the Reticuline Scaffold for New Drugs Discovery.

A thesis submitted in fulfilment of the requirements for the award of the degree of

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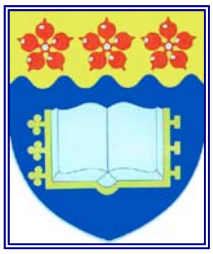

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Declaration

I, Tam-Dan (Uta) Batenburg-Nguyen hereby declare that all materials presented in this thesis, submitted in the fulfillment of the requirements for the award of Doctor of Philosophy, in the Department of Chemistry, University of Wollongong, are exclusively of my own work. These materials have not been submitted for qualifications at any other academic institution, unless otherwise referenced or acknowledged.

Tam-Dan (Uta) Batenburg-Nguyen

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List of Abbreviations.

α_1, α_2 receptor	Alpha adrenoceptors
A_1, A_{2A}, A_3	Adenosine receptors
Ag_2CO_3	Silver carbonate
AgOAc	Silver acetate
$AgOCOCF_3$	Silver trifluoroacetate
Ag_3PO_4	Silver phosphate
APV	Amprenavir
ATPase	Adenosine 5´-Triphosphatase
AT_1 receptor	Angiotensin receptor
AZT	Azidothymidine
β_1 receptor	Beta adrenoceptor
B_2 receptor	Bradykinin receptor
BBI	Bisbenzylisoquinoline
Boc	*tert*-Butoxycarbonyl group
n-BuLi	*n*-Butyl lithium
BZD receptor	Benzodiazepine receptor
CS	(*S*)-Canadine synthase
CC_{50}	Cytotoxic concentration (the concentration that was required to reduce cell growth by 50 %)
CCK receptor	Cholecystokinin receptor
$CDCl_3$	Deuterochloroform
CH_3CN	Acetonitrile
CH_3OH	Methanol

CI^+	Chemical Ionisation
CM	Cross metathesis
CNS	Central Nervous System
CXCR2, CCR1	Chemokine receptors
COR	Condeinone reductase
CNMT	(*S*)-Coclaurine-*N*-methyltransferase
gCOSY	Correlated Spectroscopy
*m*CPBA	*meta*-Chloroperoxybenzoic acid
CuI	Copper Iodide
CYP80P	Cytochrome P_{450}-dependent hydroxylase
d	Days
D1, D2S receptors	Dopamine receptors
DA transporter	Dopamine transporter
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
DDC	2,3'- Dideoxycytidine
DMAP	4-Dimethylaminopyridine
DMF	*N*,*N*-Dimethylformamide
DMG	*N*,*N*-Dimethylglycine
DOP receptor	Delta opiate receptor
DPPP	1,3-Bis(diphenylphosphino)propane
E. coli	*Escherichia coli*
EC_{50}	Effective concentration (the concentration of an agonist that produces 50 % of the maximum possible response for that agonist)

EDCI	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide
E. faecalis	*Enterococcus faecalis*
EFV	Efavirenz
EI^+	Electron impact
ESMS	Electrospray mass spectrometry
ES^+	Electrospray (positive ion mode)
ET_A receptor	Endothelin receptor
Et_3N	Triethylamine
EtOAc	Ethyl acetate
GFP	Green fluorescent protein
GABA receptor	Gamma-amino butyric acid receptor
GAL_2 receptor	Galanin receptor
h	Hour
H1, H2 receptors	Histamine receptors
HBr	Hydrogen bromide
HCl	Hydrochloric acid
HIV	Human Immunodeficiency Virus
HOAc	Acetic acid
HOBt	1-Hydroxy-1H-benzotriazole
HRMS	High resolution mass spectrometry
gHMBC	Heteronuclear Multiple Quantum Correlation
gHSQC	Heteronuclear Single Quantum Correlation
5HT receptors	5-Hydroxytryptamine, serotonin receptors
HIV-tat	Human Immunodeficiency Virus-transactivator

IC_{50}	Inhibitory concentration (the concentration required to inhibit cell growth by 50 %)
K_2CO_3	Potassium carbonate
KF	Potassium fluoride
$K_2OsO_4.2H_2O$	Potassium osmate-dihydrate
KOP receptor	Kappa opiate receptor
$LiAlH_4$	Lithium aluminium hydride
μM	Micromolar
M_2, M_3 receptors	Muscurinic receptors
MDR	Multiple-Drug Resistance
min	Minutes
ML_1 receptor	Melatonin receptor
MOP receptor	Mu opiate receptor
MRSA	Methicillin-resistant *Staphylococcus aureus*
NADPH	Nicotinamide adenine dinucleotide phosphate
$NaHCO_3$	Sodium bicarbonate
$NaBH_4$	Sodium borohydride
$NaCNBH_3$	Sodium cyanoborohydride
Na_2EDTA	Disodium ethylenediaminetetraacetic acid
$NaIO_4$	Sodium metaperiodate
nM	Nanomolar
NaOH	Sodium hydroxide
NaOAc	Sodium acetate
NE receptor	Norepinephrine receptor
NH_3	Ammonia

NIS	*N*-Iodosuccinimide
NK_3 receptor	Neurokinin receptor
NMO	*N*-Methylmorpholine *N*-oxide
NMP	*N*-Methyl-2-pyrrolidinone
NMR	Nuclear magnetic resonance
NT1 receptor	Neurotensin receptor
NVP	Nevirapine
6OMT	(*S*)-Norcoclaurine-6-*O*-methyltransferase
4'OMT	4'-*O*-Methyltransferase
ORL1 receptor	Opiate receptor-like receptor
P.aeruginosa	*Pseudomonas aeruginosa*
Pet. Spirit	Petroleum Spirit
$Pd(OAc)_2$	Palladium acetate
Pd/C	Palladium on activated carbon
$PdCl_2$	Palladium chloride
PGP	P-Glycoprotein
PPh_3	Triphenylphosphine
PRD	Pharmaceutical Research and Developement
PTLC	Preparative thin layer chromatography
RCM	Ring closing metathesis
R_f	Retention factor
RISC	RNA-induced silencing complex
RNA	Ribonucleic acid
RNAi	Ribonucleic acid interference
hpRNAs	Hair-pin ribonucleic acid

siRNAs	Small interfering ribonucleic acid
$RuCl_3.3H_2O$	Ruthenium trichloride trihydrate
ROM	Ring opening metathesis
RT	Room temperature
S. aureus	*Staphylococcus aureus*
SI	Selective Index (CC_{50}/EC_{50})
SMT	(*S*)-Scoulerine-9-*O*-methyltransferase
$SOCl_2$	Thionyl chloride
SST receptor	Somatostatin receptor
STOX	Tetrahydroberberine oxidase
N-TFA	*N*-trifluoacetyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Trimethylsilyl or tetramethylsilane (NMR)
Ts	*p*-Toluenesulfonyl
TsOH	*p*-Toluenesulfonic acid
V1a receptor	Vasopressin receptor
VIP1 receptor	Human vasoactive intestinal peptide receptor
Y1, Y2 receptors	Hypothalamic neuropeptide receptors

Abstract.

This thesis examines the synthesis of a library of benzyl- and bisbenzylisoquinolines (BBI) derivatives based on the reticuline motif. These compounds were assessed for their; i) cytotoxicity on 3 cancer cell lines, ii) activity on HIV-infected cells, iii) antibacterial activity, and iv) CNS receptor binding affinities.

Chapter 2 describes the employment of palladium-catalysed Stille, Heck and Sonogashira coupling reactions to synthesise a library of BBI derivatives. 2'-Vinyl- (**67**), 2'-allyl- (**68)** and 2'-iodo (**58**) derivatives of racemic, *N*-TFA protected, norlaudanosine were used as the key building blocks in this investigation. The key 2'-vinyl- and 2'-allyl-norlaudanosine derivatives **67** and **68**, respectively were readily prepared from palladium-catalysed Stille coupling reactions of the 2'-iodonorlaudanosine derivative **58** and vinyl- or allyl-tributylstannane. The Heck coupling reactions between the 2'-vinyl-norlaudanosine derivative **67** and the 2'-iodo-norlaudanosine derivative **58** gave not only the desired stilbene BBI derivative **65** but also the unexpected 1,1-disubstituted regioisomer **69**. This unexpected regioisomer was a result of the electron rich nature of both stating materials that favoured a cationic palladium intermediate. The best Heck coupling reaction conditions involved the use of $Pd(OAc)_2$, DMG, NaOAc and NMP at 130 ^{0}C. These conditions gave the highest yield and the best regioisomer selectively in favour of the BBI derivative **65**. Fortunately these regioisomers were readily separated by triturating the product mixture with methanol. The Heck coupling reaction between the 2'-allylnorlaudanosine derivative **68** and the aryl iodide **58** successfully afforded the three carbon tethered BBI derivative **66** in moderate yield.

It was found, however, that these Heck coupling reaction conditions were only efficient with aryl iodide precursors. This was evident from the attempted

intramolecular Heck coupling reactions on the aryl bromide precursor **89,** to give the macrocyclic BBI derivative **88**. The optimised Heck coupling reaction conditions failed to produce the desired product, while more traditional Heck coupling conditions gave the required product in poor yield (15 %).

The unsaturated BBI derivative **65** and its regioisomer **69** were subjected to hydrogenation conditions over Pd/C under a hydrogen atmosphere. However, the regioisomer **69** was found to be too sterically hindered and did not undergo the hydrogenation reaction, while derivative **65** encountered solubility problems and only *rac*-**65** underwent the hydrogenation reaction to give *rac*-**80**, leaving the less soluble *meso*-**65** intact. The compounds *rac*-**80** and *meso*-**65** were readily separated by column chromatography.

Chapter 2 also described the successful synthesis of the targeted acetylinic BBI derivative **63** *via* coupling of the 2'-ethynylbenzylisoquinoline derivative **84** with the aryl iodide **58**, using a Pd/Cu catalysed Sonogashira coupling reaction, followed by *N*-TFA deprotection of the *N*-TFA 2'-ethynylbenzylisoquinoline derivative **83**.

The synthesis of a library of 2'-arylvinyl- and 2'-arylallyl-benzylisoquinolines derivatives using the optimised Heck coupling reaction conditions developed in Chapter 2 is described in Chapter 3. This set of compounds included benzylisoquinolines having either an exocyclic *N,N*-dimethylamino (**92-103**) or *N*-acetamido (**104-107**) substituent. A third group of compounds (**108-111**) in this set had the exocyclic amino or amido group completely excluded. It was found that the Heck coupling reaction of the 2'-vinyllaudanosine derivative **67** and the aryl iodides **118, 119, 131** and **135** afforded only one regioisomer, unlike the Heck coupling between **67** and **58** in Chapter 2, which gave the two regioisomers **65** and **69**. The Heck coupling reactions between the 2'-allyllaudanosine derivative **68** and the aryl iodides **118, 119, 131** and **135** gave two

regioisomers **115a,b**; **116a,b**; **129a,b** and **137a,b,** respectively, due to two possible sites of palladium hydride elimination.

In Chapter 4, the use of the ruthenium-catalysed CM and RCM reactions toward the successful synthesis of the four carbon tethered BBI derivatives, **138-142**, in both unsaturated and saturated forms (*via* hydrogenation reactions) was described. The synthesis of the analogous two and three carbon tethered BBI derivatives *via* this method proved less efficient.

Chapter 5 reported the synthesis of a library of aminoalkyl benzylisoquinoline derivatives, incorporating both cyclic and acyclic amines (**155-162**). These analogues were obtained by a simple reductive amination methodology involving the reaction of commercially available amines with the aldehydes **186** and **187**, which were generated from the 2'-vinyl- and 2'-allyllaudanosine derivatives **67** and **68**, respectively. The initially planned pathway to one of these aldehydes involved the rearrangement of the epoxide **188,** however this epoxide was too unstable under the reaction conditions and readily underwent ring opening with *m*-chlorobenzoic acid. An alternative pathway using oxidative cleavage of the diols **190** and **191**, which were generated from dihydroxylation of the 2'-vinyl- and 2'-allyllaudanosine derivatives, **67** and **68**, respectively, was found to be more successful for the synthesis of these aldehydes.

Chapter 5 also described the synthesis of an additional class of aminoalkyl benzylisoquinoline derivatives, **163** and **164**, containing a β-amino alcohol moiety. Retro-synthetic analysis showed two possible synthetic pathways which were either *via* the ring opening of the cyclic sulfates **195** and **196** or *via* the nucleophillic displacement of the tosylates **197** and **198** with an amine nucleophile. The latter pathway proved more successful and afforded the β-amino alcohol derivatives **163** and **164**, however, the yields of these reactions should be optimised in future studies.

The synthesis of the benzylisoquinoline derivatives containing a nine- and ten-membered heterocylic ring, **165-167**, was also described in Chapter 5. The synthesis of these analogues was initially attempted *via* the intramolecular reductive amination reaction between an aldehyde moiety at the C2' position of **219** and its free isoquinoline amino group. However, the synthesis of the aldehyde moiety *via* the hydrolysis of its protected diacetal form was very difficult; therefore an alternative synthesis was developed. This method involved an intramolecular nucleophilic displacement of the chloride of the α-chloroacetamides **214** and **215** by the free isoquinoline amino moiety. This method successfully afforded the nine- and ten-membered ring benzylisoquinoline derivatives **165** and **167** in moderate yields (42-57 %). Lithium aluminium hydride reduction of **165** gave the corresponding cyclic diamino derivative **166** in high yield.

Some of the benzyl- and bis-benzylisoquinoline derivatives reported in Chapters 2-5 were sent for biological testing for their cytotoxicity on 3 cancer cell lines, activity on HIV-infected cells, their antibacterial activity and CNS receptor binding affinities. The BBI derivatives showed higher activity on cancer cell lines than the corresponding benzylisoquinoline derivatives. Various BBI and benzylisoquinoline derivatives have showed promising CNS-receptor binding affinities, especially for 5HT receptors and more prominently on the 5-HT_{1B}, 5-HT_{2A} and 5-HT_{7} receptors. At this stage, a clear structure-activity trend could not be discerned and the mode of action of these analogues was not clear. Further results on the awaiting analogues may help to develop pharmacophore models for CNS active compounds in the future, and eventually, allow the design and development of more selective and potent ligands.

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“ The most perfect form of encouragement is the response that tells the other person that you care”- Stephanie Dowrick.

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“ The difficult situations give us an unparalleled chance to growth. You don’t need to seek them out; they will find you. Rise up to meet them.”- Stephanie Dowrick.

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“ Listen to the teachings of your hearts. At the end of each day, find something to be thankful for. Give thanks. Sleep in peace.”- Stephanie Dowrick.

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It’s easy to be pleasant and gracious when things go our ways. The challenging of maturity is to be pleasant and gracious when things do not go our way.”- Stephanie Dowrick.

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